

## Failure to Connect: Experimental Evidence that Benzo(a)pyrene Impedes Embryonic Implantation

Charles W. Schmidt

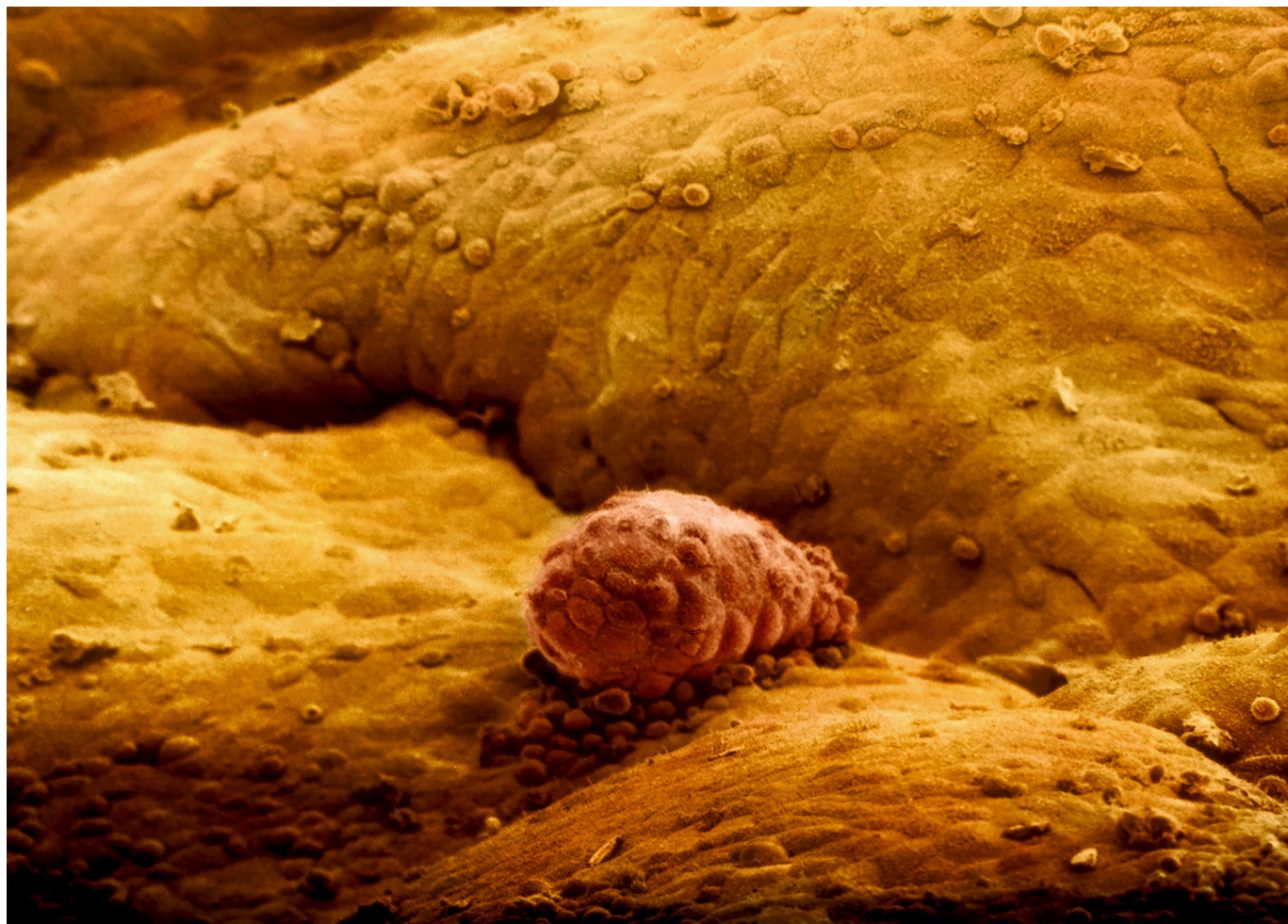
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Globally, about 1 in 10 people will experience a miscarriage at some point during their childbearing years.<sup>1</sup> These traumatic events are associated with risk factors such as infections, fetal chromosomal abnormalities, maternal age, and certain environmental exposures.<sup>2</sup> But their causes are often unclear, and that is especially true for recurrent miscarriage, which is defined as two or more pregnancy losses experienced by the same person.<sup>3</sup> About half of all recurrent miscarriages have unknown origins,<sup>4</sup> and a better understanding of how they occur is an important priority for maternal and child health researchers.<sup>1</sup> Now, experimental research in *Environmental Health Perspectives* sheds light on how one ubiquitous pollutant, benzo(a)pyrene [B(a)P], might elevate risk for recurrent miscarriage.<sup>5</sup>

A by-product of incomplete combustion, B(a)P is a polycyclic aromatic hydrocarbon (PAH) found in tobacco and wood smoke, car exhaust, and grilled foods, among other sources.<sup>6</sup> According to the

new study,<sup>5</sup> maternal exposure to B(a)P increased the expression of certain genes and proteins that may interfere with the attachment of a very early stage embryo—called a blastocyst—to the uterine wall. The research was led by Huidong Zhang, a professor at the Eighth Affiliated Hospital, Sun Yat-sen University, in Shenzhen, China.

The current experimental work builds on evidence from epidemiological and animal studies associating BaP with higher miscarriage risk.<sup>7–9</sup> During the new study, Zhang and colleagues focused specifically on developmental effects of the ultimate metabolite of B(a)P: benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE).<sup>5</sup> Previous research by this team and others showed that BPDE inhibits the normal biology of cells called trophoblasts, which form the outer layer of the blastocyst and give rise to the placenta.<sup>10,11</sup> For the developing embryo to establish a blood supply, trophoblasts must first migrate to and then invade the endometrial



The new study found that BPDE, a metabolite of benzo(a)pyrene, interfered with processes involved in embryonic implantation in mice. In people, such interference could lead to miscarriage or other pregnancy complications, according to the authors. This scanning electron micrograph shows an 8-day-old human embryo implanted in the uterus. Note: BPDE, benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide. Image: © Lennart Nilsson/Science Photo Library.

lining of the uterus.<sup>12,13</sup> But BPDE-exposed trophoblasts have exhibited a diminished capacity for migration and invasion,<sup>14</sup> “and that increases the risk of miscarriage,” says Kelly Bakulski, an assistant professor of epidemiology at the University of Michigan’s School of Public Health in Ann Arbor, who was not involved in the study.

Zhang and colleagues focused specifically on how BPDE disrupts the activity of long noncoding RNAs (lncRNAs),<sup>5</sup> which have key roles in trophoblast functioning. In an earlier cell study, the team identified 22 lncRNAs that are more active in human trophoblasts after BPDE exposure.<sup>11</sup> But how lncRNAs might be involved in BPDE’s inhibition of trophoblast migration and invasion was not clear.

The team used a novel lncRNA, called *lnc-HZ09*, that they identified in this study.<sup>5</sup> For the first set of experiments, they engineered two sets of human trophoblast cell lines, one in which *lnc-HZ09* was overexpressed and another in which it was silenced. Results showed that the cells that overexpressed *lnc-HZ09* were less able to move normally through a gel matrix, “suggesting that trophoblasts with high levels of *lnc-HZ09* may have similar difficulties migrating and invading into uterine tissues,” Bakulski says. Furthermore, these cells contained abnormally low levels of three proteins suspected of playing key roles in trophoblast migration and invasion: PLD1, RAC1, and CDC42. The cells in which *lnc-HZ09* was silenced showed “significantly greater migration and invasion,” the authors wrote.

Next, the researchers analyzed placental tissues collected from two groups of Chinese women: 15 who had experienced unexplained recurrent miscarriages, and a control group of 15 who had undergone abortions. The results supported the role of *lnc-HZ09*: Compared with tissues from the control group, samples collected from women who miscarried had higher *lnc-HZ09* gene expression levels, as well as higher levels of PBDE-DNA adducts, the sites where the chemical attached to DNA.

For a final experiment, the team treated pregnant mice with B(a)P at doses of 0.05 or 2.0 mg/kg body weight to induce miscarriage. The researchers found that in the animals receiving the higher dose, levels of PLD1, RAC1, and CDC42—the three proteins observed in the human trophoblast cell experiment—were lower in placental tissues.

According to the authors, the results suggest that *lncHZ09* suppressed trophoblast cell migration and invasion through its effects on the PLD1/RAC1/CDC42 pathway. “We believe that the combination of these experiments suggests that, in humans, B(a)P/PBDE exposure may induce miscarriage,” Zhang says.

Ping-Kun Zhou, president of the Chinese Society of Toxicology and a professor at the Beijing Institute of Radiation Medicine, agrees this is an interesting finding. “The molecular mechanism might be further verified using primary trophoblast cells from villous [placental] tissues from individuals experiencing a miscarriage,” says Zhou, who was not involved in the study. In the meantime, Zhou adds, pregnant women should reduce their exposure to PAHs as much as possible by, for example, avoiding smoking, supporting adoption of

clean energy, eating steamed rather than fried food, and ensuring their gas stove vents properly.

**Charles W. Schmidt**, MS, is an award-winning journalist in Portland, ME, whose work has appeared in *Scientific American*, *Nature*, *Science*, *Discover Magazine*, *Undark*, *The Washington Post*, and many other publications.

## References

- Ha S, Ghimire S, Martinez V. 2022. Outdoor air pollution and pregnancy loss: a review of recent literature. *Curr Epidemiol Rep* 9(4):387–405, <https://doi.org/10.1007/s40471-022-00304-w>.
- Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, et al. 2021. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. *Lancet* 397(10285):1658–1667, PMID: 33915094, [https://doi.org/10.1016/S0140-6736\(21\)00682-6](https://doi.org/10.1016/S0140-6736(21)00682-6).
- American Society for Reproductive Medicine. 2016. What is recurrent pregnancy loss (RPL)? <https://www.reproductivefacts.org/news-and-publications/patient-fact-sheets-and-booklets/documents/fact-sheets-and-info-booklets/what-is-recurrent-pregnancy-loss-rpl/> [accessed 12 April 2023].
- ESHRE Capri Workshop Group. 2008. Genetic aspects of female reproduction. *Hum Reprod Update* 14(4):293–307, PMID: 18385259, <https://doi.org/10.1093/humupd/dmn009>.
- Dai M, Huang W, Huang X, Ma C, Wang R, Tian P, et al. 2023. BPDE, the migration and invasion of human trophoblast cells, and occurrence of miscarriage in humans: roles of a novel *lncRNA-HZ09*. *Environ Health Perspect* 131(1):17009, PMID: 36719213, <https://doi.org/10.1289/EHP10477>.
- IARC (International Agency for Research on Cancer). 2012. Chemical agents and related occupations. IARC Monogr Eval Carcinog Risks Hum. 100(pt F): 9–562, PMID: 23189753.
- Neal MS, Zhu J, Holloway AC, Foster WG. 2007. Follicle growth is inhibited by benzo[*a*]pyrene, at concentrations representative of human exposure, in an isolated rat follicle culture assay. *Hum Reprod* 22(4):961–967, PMID: 17218370, <https://doi.org/10.1093/humrep/del487>.
- Patashekas J, Ciunione E, Barkiene M, Zurlyte I, Jonauskas G, Sliachtic N, et al. 1996. Environmental and health monitoring in Lithuanian cities: exposure to heavy metals and benz(a)pyrene in Vilnius and Siauliai residents. *J Environ Pathol Toxicol Oncol* 15(2–4):135–141, PMID: 9216796.
- Zhao Y, Chen X, Liu X, Ding Y, Gao R, Qiu Y, et al. 2014. Exposure of mice to benzo(a)pyrene impairs endometrial receptivity and reduces the number of implantation sites during early pregnancy. *Food Chem Toxicol* 69:244–251, PMID: 24769007, <https://doi.org/10.1016/j.fct.2014.04.021>.
- Xu Z, Tian P, Guo J, Mi C, Liang T, Xie J, et al. 2021. Lnc-HZ01 with m6A RNA methylation inhibits human trophoblast cell proliferation and induces miscarriage by up-regulating BPDE-activated Lnc-HZ01/MXD1 positive feedback loop. *Sci Total Environ* 776:145950, PMID: 33647641, <https://doi.org/10.1016/j.scitotenv.2021.145950>.
- Liang T, Xie J, Zhao J, Huang W, Xu Z, Tian P, et al. 2021. Novel Lnc-HZ03 and miR-hz03 promote BPDE-induced human trophoblastic cell apoptosis and induce miscarriage by upregulating p53/SAT1 pathway. *Cell Biol Toxicol* 37(6):951–970, PMID: 33566220, <https://doi.org/10.1007/s10565-021-09583-3>.
- Kliman HJ. 1998. From Trophoblast to Human Placenta. In: *The Encyclopedia of Reproduction*, Vol. 4. Knobil E, Neill JD, eds. 1st ed. United Kingdom: Elsevier Science, Vol. 4. [https://medicine.yale.edu/obgyn/kliman/placenta/research/trophoblast%20to%20placenta%20eor\\_163163\\_284\\_18220\\_v1.pdf](https://medicine.yale.edu/obgyn/kliman/placenta/research/trophoblast%20to%20placenta%20eor_163163_284_18220_v1.pdf) [accessed 12 April 2023].
- Knöfler M, Haider S, Saleh L, Pollheimer J, Gamage TKJB, James J, et al. 2019. Human placenta and trophoblast development: key molecular mechanisms and model systems. *Cell Mol Life Sci* 76(18):3479–3496, PMID: 31049600, <https://doi.org/10.1007/s00018-019-03104-6>.
- Ye Y, Jiang S, Zhang C, Cheng Y, Zhong H, Du T, et al. 2020. Environmental pollutant benzo(a)pyrene induces recurrent pregnancy loss through promoting apoptosis and suppressing migration of extravillous trophoblast. *Biomed Res Int* 2020:8983494, PMID: 33123590, <https://doi.org/10.1155/2020/8983494>.